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Chapter 6

Observations of a polyclad flatworm affecting acroporid corals in captivity

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ABSTRACT

In 2003 and 2004, the Birch Aquarium at Scripps lost two of its large *Acropora* spp. colonies. Both had resided in an Indo-Pacific reef tank for over eight years and spawned a number of times. Flatworms were isolated from one of these corals, *Acropora valida* (Dana, 1846). The worms were nearly transparent and had a multibranched gut containing intact zooxanthellae, which provided excellent camouflage against the coral's tissue. Infected coral fragments were exposed to chemical and physical treatments to attempt to eradicate the flatworms but recommended concentrations of various treatments did not eliminate them. Increasing concentrations or extending the treatment period did help to eradicate the flatworms but may have also killed the corals. The flatworm was tentatively identified as *Apidioplana* sp. (Bock, 1926), a polyclad found on surfaces of corals. Because there is very little information about the genus *Apidioplana*, some of its life history was inferred from common characteristics in the order Polycladida. The lack of information in the literature as well as from communication with coral biologists suggests that this particular flatworm may not pose a threat to corals in the wild. There may be a natural predator that keeps their harmful effects in check, but this has yet to be identified and introduced in aquaria. Based on observations presented here and by other aquarists, some possible quarantine and treatment protocols are presented.

INTRODUCTION

In recent years, aquarists have encountered a flatworm (Phylum Platyhelminthes) that eats the tissue of *Acropora* spp. corals (Figure 1). In aquarium literature they are commonly referred to as "*Acropora*-eating flatworms" (Sprung 2001) or "AEFW". They are capable of killing an *Acropora* colony and have become a nuisance to the reef aquarium. Unlike flatworms such as *Waminoa* sp. (Convolutidae) or *Convolutriloba* sp. (Convolutidae) that can be relatively easy to eliminate through the use of chemicals such as Levamisole (Delbeek and Sprung 2005) and Salifert's Flatworm Exit® (www1) or biological control such as *Chelidonura varians or Halichoeres* spp. (Sprung and Delbeek 1997, Delbeek and Sprung 2005, Schiemer 2002), the *Acropora*-eating flatworms seem more resistant to chemical treatments and an effective biological control has not yet been identified.

In September 2003 and June 2004, the Birch Aquarium at Scripps (BAS) lost two large exhibit corals, *Acropora* sp. "brown"(house name) and *Acropora valida*, respectively. Both colonies were collected in Fiji (Paul Votava, Pers. comm.) and purchased by the BAS from a wholesaler in February 1995. The cause of death in *Acropora* sp. "brown" was not known until after the colony (8 kg, 1 m diameter) was removed from the exhibit tank. Upon close observation, lower portions of the skeleton were found to have flatworm egg cases attached to them (Figure 2).

Given this observation, closer attention was paid to the *A. valida* colony. Branches in the colony that appeared pale in comparison to healthy areas were removed and observed under a dissecting microscope. Well-camouflaged flatworms were observed over the coral's tissue (Figure 1). Apparently healthy portions

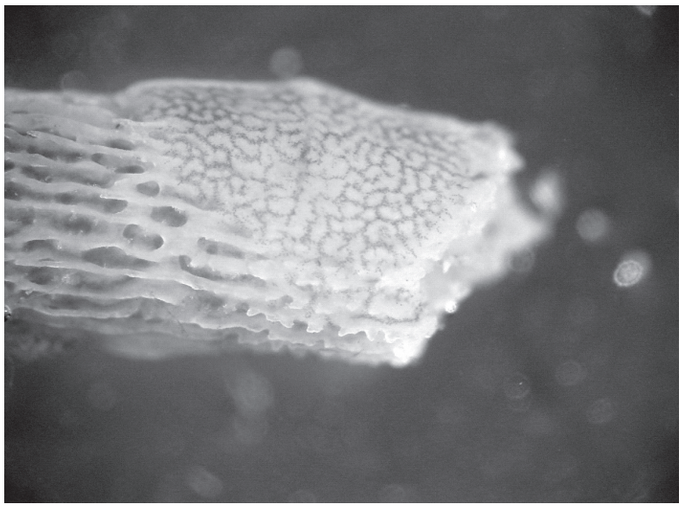

*Figure 1: An "*Acropora*-eating flatworm" gliding over* A. valida *branch tip. This specimen was relatively easy to locate as it was sitting over the branch tip.*

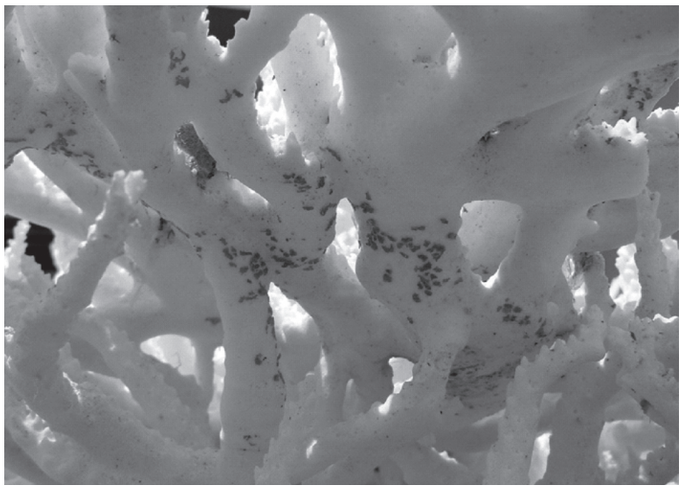

Figure 2: Flatworm egg cases attached to the undersides of branches of Acropora *sp.*

of the colony were removed for treatment to prevent further flatworm damage. In hopes of saving fragments from this colony, two different treatments were applied: lowered salinity (22 ppt for 21 min, 15 ppt for 3 min, 12 ppt for 3 min and 6 ppt for 0.5 min) and Levasole baths (4.0 $mg.L^{-1}$ for 1 h and 8.0 $mg.L^{-1}$ for 1 h). Both treatments were administered in haste and there was no experimental design or planning of treatments. In January 2007, identical flatworms were obtained from Atlantis Marine World (AMW Long Island, NY). These specimens were preserved for DNA sequencing and anatomical study. The flatworms have been tentatively identified as *Apidioplana* sp. (Order Polycladida) based on photos and descriptions in Newman and Cannon (2003). Because the flatworms discussed in this paper have not been positively identified here, they will be referred to by their common name, *Acropora*-eating flatworms, or AEFWs.

Literature and Internet searches provided very little information about *Apidioplana* sp. Two coral biologists were contacted but were unfamiliar with *Apidioplana* sp. Given the generally poor understanding of this species, the goals of this paper are to help the aquarist become familiar with the symptoms characteristic of this coral parasite and to provide a possible quarantine regimen and treatment methods for specimens on display.

METHODS

Observation

Live flatworms alone and on coral tissue were observed through a dissecting microscope

(Bausch & Lomb, P&L 0.7x- 3x). Further observations of flatworms isolated from corals were made by compound miscroscope (Meiji Techno Co., Japan 10x – 40x). Photos (Sony DSC-P72) and video (Meiji Techno Color Video Camera with JVC VHS recorder) of specimens were taken through both scopes.

Locating flatworms

Pale colored fragments were removed from the *A. valida* colony, as these were the suspected areas of infection. The fragments were observed under a dissecting scope. A slightly raised area on the coral tissue was usually indicative of a flatworm. These sections also appeared somewhat lighter in color than the surrounding coral tissue. By pulsing jets of water to this area with a pipette, one could test whether a worm was present or not (Figure 3). The pulse of water would stimulate movement or lift a part of the worm's body off of the coral. A number of flatworms were isolated and collected in this manner.

Treatments

Apparently healthy portions of the *A. valida* colony were fragmented and placed into a 76 L glass aquarium. The treatment tank was filled with water from the exhibit tank where *A. valida* resided. Tank temperature (25 °C), was maintained by a submersible heater with a built-in thermostat. The tank was lit with a 250 W metal halide bulb approximately 51 cm above the tank. A small submersible pump provided circulation and the tank was aerated using an air stone. No filtration was employed in the treatment tank. After each treatment, 50-75 % water changes were performed using water from the tank where *A. valida* resided. Treatments included Levasole (levamisole hydrochloride, Schering-Plough Animal Health Corp. New Jersey, USA, 4.0 $mg.L^{-1}$ for 1 h and 8.0 $mg.L^{-1}$ for 1 h, Table 1) and lowered salinity (22 ppt for 21 min, 15 ppt for 3 min, 12 ppt for 3 min and 6 ppt for 0.5 min, Table 2). Buffered (Warner Marine Research, USA), filtered freshwater (SpectraPure, Inc. Part # TM0050-001-CSP-DI. Arizona, USA) was used to lower salinity. The freshwater was also heated to the temperature of the treatment tank.

Sample preservation

Flatworms infecting the *A.valida* colony at BAS were not preserved for further study. In January 2007, Joe Yaiullo of AMW sent *A. pulchra* fragments, infected with AEFW, to the BAS. A number of these specimens were preserved in frozen 4% paraformaldehyde in buffer, which was allowed to slowly thaw. Samples were stored for future morphological study.

RESULTS

Description of AEFWs

The specimens from BAS and AMW appeared to be identical. They averaged 5 mm in length, with at least one individual measuring 10 mm long. Body shape was oval when a flatworm was at rest but could change, conforming to coral morphology. Cilia were present along the edge of the body (Figure 5). Dark eyespots (photoreceptors) were located anterio-dorsally. The worms were almost transparent except for a

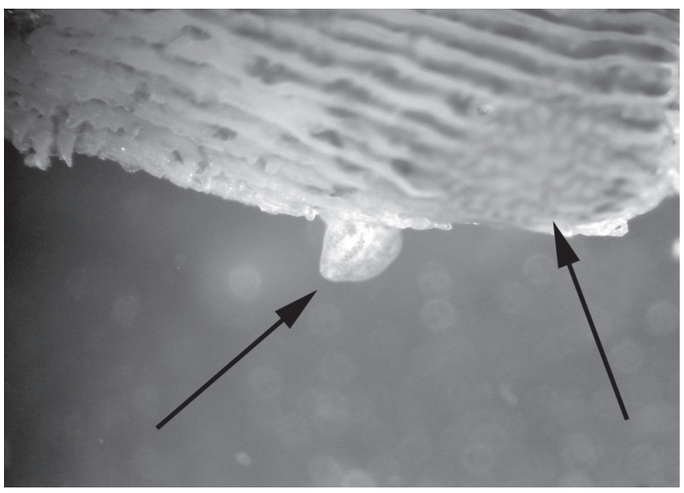

Figure 3: Left arrow: flatworm rising off coral tissue after pipetting a jet of water toward it. Right arrow: a second flatworm camouflaged on coral tissue.

reticulate pattern of brown lines (multi-branched gut). Intact zooxanthellae were visible within the multi-branched gut (Figure 6). Based on these morphological observations, the flatworm was tentatively identified as *Apidioplana* sp., an acotylean polyclad flatworm (Phylum Platyhelminthes) that was "recently found on hard corals" (Cannon and Newman, 2003).

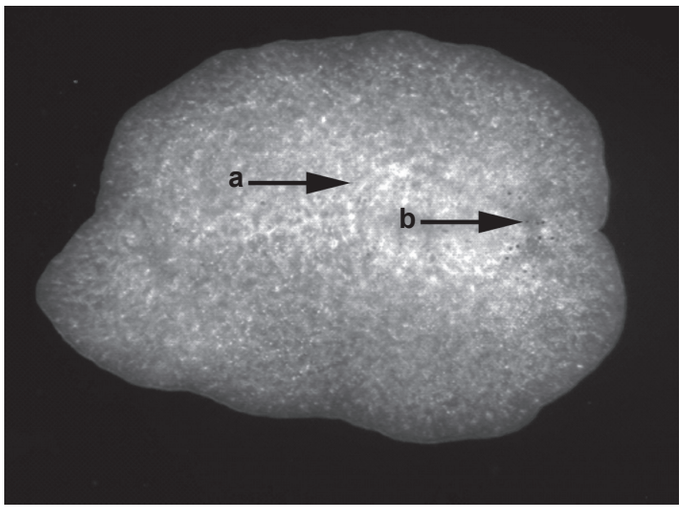

Figure 4: Dorsal view of AEFW viewed under dissecting scope. a. multi-branched gut (light grey lines), b. photoreceptors (dark spots) at anterior (right) end of body (Photo: G. Rouse).

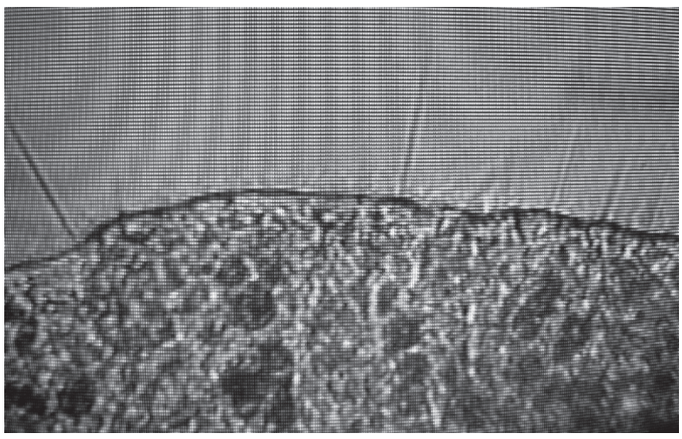

Figure 5: Cilia visible at the edge of the flatworm body.

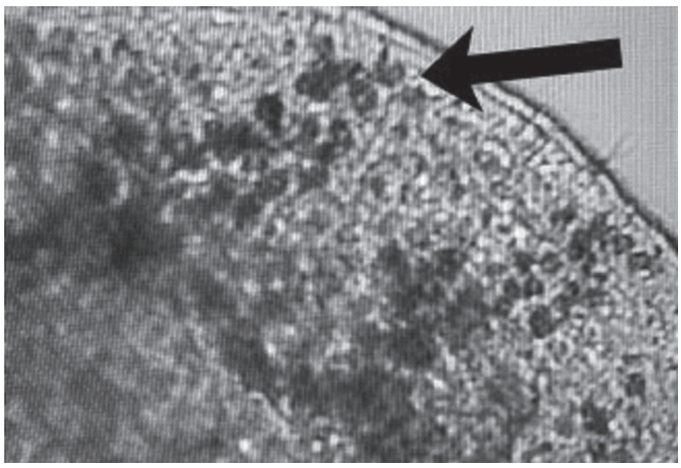

Figure 6: 400 X magnification of the edge of an AEFW specimen. Arrow: intact zooxanthellae within the flatworm's multibranched gut. This gave the worm an overall brown color.

Description of AEFW eggs

AEFW eggs on BAS and AMW coral branches were arranged in tightly massed groups. The eggs were brown and approximately 0.25 mm in diameter. Where eggs were found, they were always at the base of the coral branches where coral tissue was absent (Figures 7 and 8).

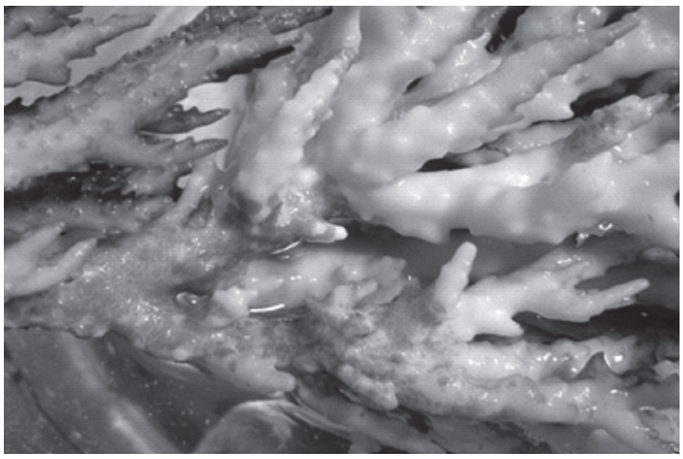

Figure 7: Branches of A. valida *with tightly packed AEFW egg masses (grey-colored portion of skeleton, lower left).*

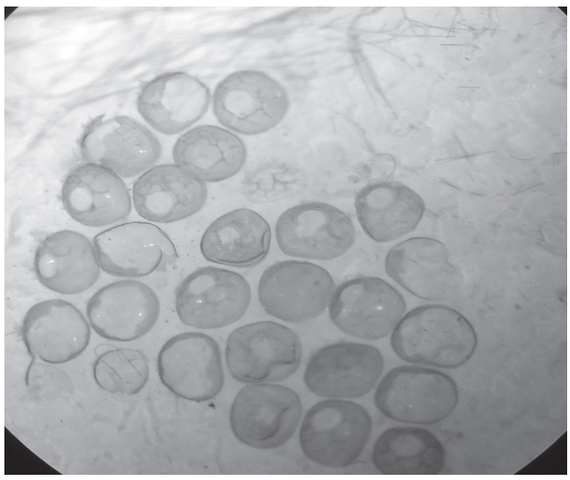

Figure 8: Close up of hatched AEFW eggs on A. valida *skeleton.*

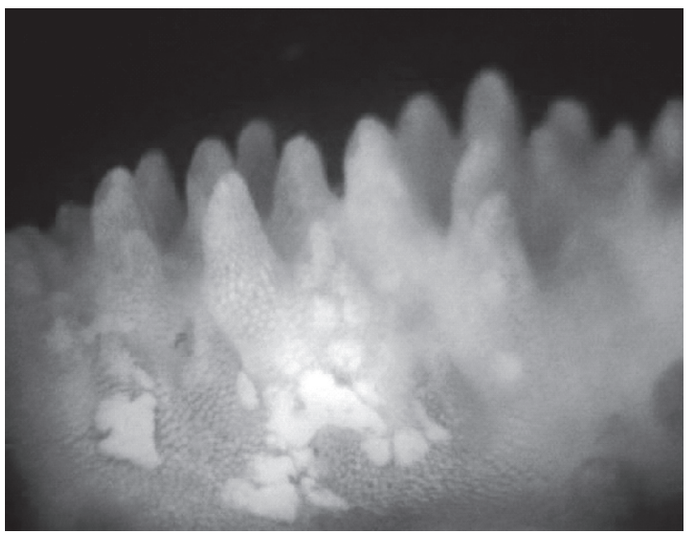

Figure 9: Feeding spots left by AEFWs on a branch of Acropora pulchra.

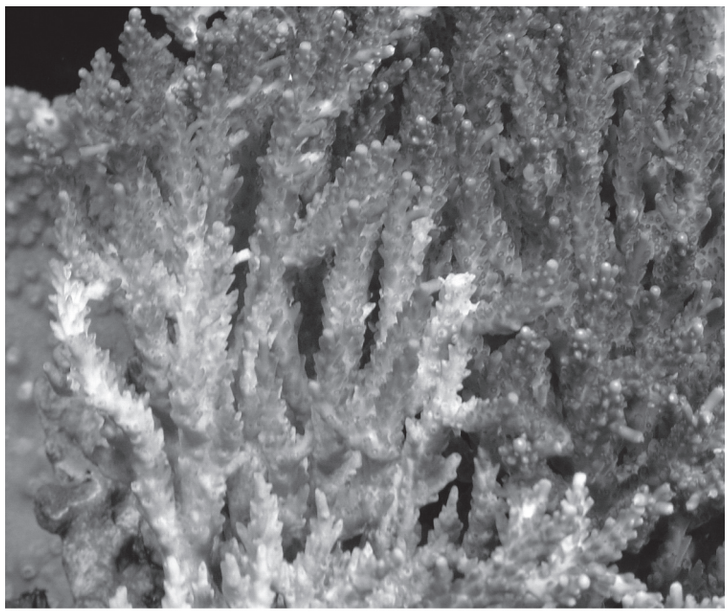

Figure 10: Pale A. valida *branches (bottom left) infected with AEFWs. Purple tips on some of the pale branches demonstrate the presence of tissue on infected branches.*

Description of symptoms

Common early signs for the presence of AEFW in *A. valida* at the BAS and *A. pulchra* from AMW were small (~1 mm), pale, circular spots near the base of branches. These pale spots, which form tracks across coral tissue, appear to be the result of AEFW feeding behavior (Figure 9). In severely infected areas, whole branches were almost white, leaving only the coral's natural pigments at branch tips (Figure 10).

Effectiveness of treatments

Infected coral fragments were first treated with Levasole at different concentrations (Table 1). The second treatment was lowered salinity (Table 2), reduced from the normal tank seawater salinity of 33.5 ppt. During both treatments, fragments were pulsed with water using a turkey baster. This action helped remove worms that were weakly attached to the coral, although many did remain attached. All fragments, whether treated with Levasole or bathed in lowered salinity water, died within 10 days.

Table 1: Results of Levasole Treatments on AEFWs. (+: Flatworms were weakened, with some falling off coral)

Levasole (mg.L^{-1})	**Duration (h)**	**Effect**
4.0	1	None
8.0	1	Minor +

Table 2: Results of Hyposalinity Treatments on AEFWs.

Lowered Salinity (ppt)	**Duration (min)**	**Effect**
22	21	None
15	3	None
12	3	None
6	0.5	Worms dropping

DISCUSSION

Species identification

Because the flatworm described in this paper has not been positively identified, only tentative conclusions can be made regarding its life history. The AEFW may share some characteristics with flatworms in the class polycladida (e.g. multi-branched gut, simultaneous hermaphrodite). Although there is some information about Apidioplanids that are found on gorgonians, very little information is available about the life history of the *Apidioplana* sp. that affects Acroporids (Newman and Cannon 2003; Naoki and Masakazu, 2003). Future DNA sequencing and anatomical studies that will be performed at SIO may result in a species name for this particular AEFW if it is within the genus *Apidioplana* (Rouse, pers. com.). At this time, the *Apidioplana* sp. found on stony corals has not been described to the species level.

It is unknown when or how the flatworms were introduced into our tank. Aquarium professionals and hobbyists who maintain reef aquariums seem to come across this worm more often than do reef biologists (www2, 3, 4, 5). For example, in Newman and Cannon (2003), one of two photos of this particular flatworm was taken by an aquarium hobbyist. Coral biologists Carden Wallace (Queensland Museum) and Nancy Knowlton (SIO) were both unfamiliar with the Acroporid-infecting *Apidioplana* sp. or a flatworm that behaves as AEFWs do. This suggests that AEFWs may not be negatively affecting Acroporid corals in the wild and are not commonly observed by field biologists. A natural predator of AEFWs in the wild, possibly commensal crabs on corals such as *Tetralia* spp. or *Trapezia* spp, may help keep corals healthy. Therefore, it appears that AEFWs are a low-impact parasite of corals in the wild, but when unchecked by their normal predators, serve as a deadly parasite of corals in captivity.

Feeding behavior

It is not yet clear whether AEFWs search specifically for zooxanthellae or eat coral tissue indiscriminately. Feeding by polyclads is accomplished through the use of a protrusible pharynx. Strong musculature at the base of the pharynx allow for great feeding ability (Purdhoe, 1985). Presumably, the AEFW would use its protrusible mouth to puncture the coral ectodermis and mesoglea and enter the gastrodermis to retrieve zooxanthellae. Most turbellarians locate food by chemoreception (Brusca and Brusca, 1990), however it is unclear whether AEFWs could locate zooxanthellae in this manner. Some coral fragments affected by AEFWs at BAS were found with only the coral's (purple) pigment present This indicates that coral tissue remained while zooxanthellae were absent, suggesting that AEFWs selectively removed zooxanthellae from the coral tissue. Alternately, it is possible that because there were fewer or no zooxanthellae at branch tips, the flatworms left these purple-colored branch tips alone. Another possibility is that the stress of AEFWs feeding induced coral tissue bleaching and the lost zooxanthellae were not directly eaten by the worm.

Zooxanthellae in the multibranched gut of AEFWs provided excellent camouflage while worms were resting on coral tissue. The presence of intact zooxanthellae within the gut may also provide another benefit in the form of a food source (photosynthates). This has not yet been examined, however conducting a Pulse Amplitude Modulation Fluorometer (PAM) test might be one way to determine whether the zooxanthellae within the AEFW gut are viable and actively photosynthesizing (Rowher, pers. com.).

Reproduction

If AEFWs are indeed Apidioplanids, some conclusions may be drawn in terms of their reproduction that may be important to the aquarist facing an AEFW infestation. Polyclad flatworms are simultaneous hermaphrodites. Eggs are laid directly on a substrate using a strong adhesive cement and hatch within a few days (Newman and Cannon, 2003).

Apidioplanids are acotylean (i.e., a division of flatworms without ventral suction disk) and most acotyleans bypass a larval stage (Newman and Cannon, 2003; Prudhoe, 1985). The young worms hatch and move directly onto the substrate. Asexual reproduction in polyclads is also possible. A fragment of tissue containing a portion of the "brain" can regenerate into a new individual (Newman and Cannon, 2003). If AEFWs do not have a free-swimming larval stage, they must migrate from one coral colony to another in a different manner. One possible strategy relies on the storage of zooxanthellae in the gut. If zooxanthellae within the flatworm's gut are active and produce photosynthates, it may be possible for the AEFW to benefit from this food source while searching for the next coral colony to feed on. This would make

AEFWs particularly difficult to remove from infested aquaria. However, if AEFWs do posses a free-living larval stage, then mechanical filtration and U.V. or ozone sterilization may be useful ways to reduce their spread.

Treatments

The treatments performed on *A. valida* fragments at the BAS were done in haste, in hopes of saving at least some of the coral colony. There was no experimental design and therefore the results of these treatments may not be reliable. Additionally, although water changes were performed in the treatment tank, biological filtration was absent and this may have added stress to the corals. However, some anecdotal observations by hobbyists (www6) support the results presented here. R. Byrnda (in Delbeek and Sprung 2006) mentions that these flatworms may be building resistance to Levamisole treatments. Other drugs commonly available for the eradication of flatworms in the genera *Convulotriloba* and *Waminoa* do not seem to be very effective against AEFWs (www6). Furthermore, higher-than-recommended doses of various drugs andtreatments have eradicated AEFWs but also seem to have deleterious effects on the treated corals (www6).

One possible method to reduce AEFW populations effectively and safely is currently being employed by Joe Yaiullo at AMW. Due to the large exhibit size (75,700 L) and the large size of *Acropora pulchra* colonies, bath treatments are not possible. Instead, filtered freshwater is passed through a pressure washer. A common garden hose attached to the pressure washer is used while Hookah diving (Surface supplied air) in the tank. A nozzle at the end of the hose allows control of the freshwater released. Freshwater is applied to suspected areas of infection (Figure 11). Currently, this fresh water wash is applied to these corals once every 4-6 weeks. This schedule seems to keep the worm population under control (Yaiullo, pers. com,). Spraying fresh water helps to loosen flatworms from the coral and move them into the water column. Various fishes (*Genicanthus* sp., *Zebrasoma* spp., *Pseudanthias* spp., *Paracheilinus* spp., *Halichoeres* spp. and *Odonus niger*) have been observed eating the displaced flatworms (Yaiullo, pers. com.). This suggests that AEFWs may not contain toxic compounds as do *Convulotriloba* sp. and *Waminoa* sp. (Delbeek and Sprung 2005), both of which are normally visible to the naked eye. AEFWs, on the other hand, seem to use camouflage

Figure 11: Joe Yaiullo of Atlantis Marine World spraying freshwater at an Acropora colony infected with AEFW (Photo: J. Yaiullo).

as their mechanism of defense. The method of spraying freshwater directly onto corals is a chemical-free treatment approach. A huge benefit to this approach is that the surrounding saltwater quickly dilutes the freshwater, thereby preventing added stress to the coral's tissue. This method could be employed on a smaller scale by siphoning freshwater with airline tubing (flexible and rigid) towards corals as a part of a quarantine regimen. Additionally, if a few *Halichoeres* spp. wrasses could be added to the quarantine tank while performing the freshwater treatments they may help eat the loosened worms. A series of Levamisole baths (M. Carl, in Delbeek and Sprung, 2005), along with the freshwater spray treatments described above, may provide an adequate treatment for the eradication of AEFWs. Detecting and removing AEFW eggs from the branches of newly acquired coral colonies could also help reduce or prevent a flatworm problem.

Host specificity

While two *Acropora* colonies at the BAS were negatively affected by AEFWs, it is interesting to note that *A. yongei* colonies in the same tank were not affected. A large colony of *A. yongei*, from a reserve tank, was used to replace the *Acropora* sp. "brown" colony that was lost. The *A. yongei* colony was positioned approximately 30 cm from the *A. valida* colony and lived next to it for 8 months. During the *A. valida* colony's infestation with AEFWs and after its removal from the tank, *A yongei* never showed signs of stress associated with an AEFW attack. *A. yongei's* morphology is an arborescent, open structure, which allows for more water flow, in contrast to *A. valida's* corymbose structure. Furthermore, *A. yongei* seems to produce copious amounts of mucous in comparison to the *A. valida* and *Acropora* sp. "brown" colonies. Its mucous and/or colony structure may have helped *A. yongei* avoid AEFW infection. However, the open structure of *A. yongei* does not explain why *A. pulchra* from AMW, with a similar morphology, could not survive infection by AEFWs. R. Byrnda (in Delbeek and Sprung, 2005) also states that the most affected *Acropora* species seem to be *A. valida* and *A. millipora* and that *A. yongei* seems to be resistant to this flatworm. Similar observations have been made by reef hobbyists (www6, 7). Thus, in addition to colony morphology, susceptibility to AEFWs may also depend on a coral's mucous production, or other inhibitory mechanisms such as mucous composition, bacterial colonization, or production of deterrent compounds (Marhaver, pers. com.). This may offer some relief to aquarists, as AEFWs may not spread beyond their target species.

ACKNOWLEDGEMENTS

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REFERENCES

Brusca, B.C. and G.J. Brusca, 1990. Invertebrates. Sinauer Associates, Inc., Sunderland, Massachusetts, USA: 922 pp.

Delbeek, J.C. and J. Sprung, 2005. The Reef Aquarium Volume Three: Science, Art and Technology. Ricordea Publishing, Florida, USA: 680 pp.

Kumagai, N.H. and M.N. Aoki, 2003. Seasonal changes in the epifaunal community on the shallow-water gorgonian Melithaea flabellifera. Journal of the Marine Biological Association of the United Kingdom, 83:1221-1222.

Newman, L. and L. Cannon, 2003. Marine Flatworms: The World of Polyclads. CSIRO Publishing, Collingwood, Australia: 97 pp.

Prudhoe, S., 1985. A Monograph on Polyclad Turbellaria. Oxford University Press, New York, USA: 259 pp.

Sprung, J., 2001. Invertebrates: A Quick Reference Guide. Ricordea Publishing, Miami, Florida, USA: 240 pp.

Sprung, J. and J.C. Delbeek, 1997. The Reef Aquarium Volume Two: A Comprehensive Guide to the Identification and Care of Tropical Marine Invertebrates. Ricordea Publishing, Florida, USA: 546 pp.

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INTERNET RESOURCES

www1. http://www.melevsreef.com/flatworms.html
www2. http://www.melevsreef.com/aefw.html
www3. http://www.reefcentral.com/forums/shorthread.php?s=&threadid=899108&perpage=25&pagenumber=1
www4. http://zoology.unh.edu/faculty/litvaitis/k-12Primer/k-12.htm
www5. http://www.korallenriff.de/acrop-girz.html
www6. http:/reefcentral.com/forums/showthread.php?s=&threaded=899108&perpage=25&pagenubmer=9
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